

Quantifying Carbon-14 for Biology Using Cavity Ring-Down Spectroscopy

A. Daniel McCartt,^{*,†} Ted J. Ognibene,[†] Graham Bench,[†] and Kenneth W.
Turteltaub[‡]

[†]*Center for Accelerator Mass Spectrometry, Lawrence Livermore National Lab, 7000 East Ave,
Livermore, CA 94550 USA*

[‡]*Biology and Biotechnology Division, Lawrence Livermore National Lab, 7000 East Ave,
Livermore, CA 94550 USA*

E-mail: mccartt1@llnl.gov

The *Guinea Pig Sample Preparation*, *Carbon Dioxide Production*, and *Carbon-14 Cavity Ring-Down Spectroscopy* sections below describe an entire suite of methods used to acquire biological carbon-14 data with a CRDS instrument.

Guinea Pig Sample Preparation.

Plasma and tissue samples were obtained from guinea pigs that were exposed to a ^{14}C -labeled intravenous (IV) dose of an oxime acetyltransferase reactivator. Male Hartley guinea pigs were maintained in AAALAC accredited animal facility and were administered a single IV dose of 100 mg/kg ^{14}C -oxime in sterile saline through a pre-inserted jugular vein catheter according to an IACUC approved animal protocol. Following dose administration, whole blood samples (approximately 0.3 mL) were collected from each animal via the jugular vein at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hours post dose, placed into Microtainer tubes coated with lithium heparin (Becton Dickinson, Franklin Lakes, NJ) and placed on ice. Plasma was separated from the whole blood by centrifugation (10,000 x g for 2 min) within 1 hour of collection, the volume recorded and stored at -20°C until analysis by CRDS and AMS. At each time-point animals were euthanized by carbon dioxide asphyxiation and tissues were excised, rinsed twice in phosphate buffered saline (PBS), placed in pre-weighed glass vials (28 x 60 mm), and immediately frozen at -20°C until analysis. Prior to analysis by CRDS the liver was homogenized using established methods¹. An aliquot of the homogenate containing 5 mg of carbon was used for analysis.

Carbon Dioxide Production.

Duplicate organic samples containing up to 5 mg carbon were aliquoted and dried under vacuum. These samples were sealed in a quartz tube containing approximately 150 mg copper oxide using an acetylene torch. The tubes were then combusted at 900C for 2 hours. Following combustion, AMS samples were graphitized and CRDS samples were transferred to the spectrometer front-end for purification. For CRDS sample purification, the quartz tubes were cracked under vacuum inside a bellows tube, and the sample gas was passed over an isopropanol/dry ice water trap. The gas was then pulled onto a liquid nitrogen cold

finger, with the remaining gaseous species evacuated. The purified carbon dioxide was then introduced into the optical cavity. Measurements were made discretely with the samples sealed for the duration of the measurement. The cold finger was placed as close to the cavity as possible to reduce sample size requirements. Approximately 20% of the carbon dioxide was used for purging the cavity and adjusting the pressure. An additional 20% of sample is wasted on oversized plumbing, a proportion that will be greatly reduced in the future.

Carbon-14 Cavity Ring-Down Spectroscopy.

The spectrometer resides on a 4x6 ft optical table. The majority of this space is occupied by a custom freezer obtained from Scientemp Corporation (Adrian, MI, USA). This freezer is oversized for the potential future accommodation of longer optical cavities. The QCL was obtained from Hamamatsu Photonics (Hamamatsu City, Shizuoka, Japan) and using custom electronics the laser linewidth was reduced to less than 3 MHz at 2209 cm^{-1} and 40 mW. Further details of the experimental setup can be found in the references²⁻⁴.

For this system, cavity ring-down spectroscopy (CRDS) is performed with a stabilized optical cavity, and the laser wavelength is modulated about the cavity's resonant frequency. The cavity is stabilized using the dynamic regions from strong transitions of carbon dioxide isotopologues⁴. The stabilized cavity's free-spectral-range is used to determine the spectral frequency axis with the etalon-based wavelength monitor only being used for binning. Spectra are taken of the cavity under vacuum and subtracted from carbon dioxide spectra to account for variations in optical cavity loss with wavenumber. A Faraday isolator is included to prevent cavity reflections and back-scatter from interfering with QCL output. Measurements of carbon dioxide from combusted biological samples are analyzed with a spectroscopic model to accurately compensate for carbon dioxide isotopologues and nitrous oxide at the $^{14}\text{C}^{16}\text{O}_2$ measurement transition location. This spectroscopic model was developed using measurements of isotopically enriched carbon dioxide to characterize interfering transitions at relevant measurement conditions². Finally, the instrument response to carbon-14 is calibrated for each experimental condition daily with measurements of sample

standards containing isotopic carbon-14 concentrations of zero and $5 \cdot 10^{-11} \text{ }^{14}\text{C}/\text{C}$.

References

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